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Figure 1A

SEQ	NO. O			<u>.</u>			
9	mouse_E3all	MASEMEPEVO	AI D- RSLLEC	SAEEI AGRWL	QAT DL NR E VY	QHLAHCVPKI	49
4	human_E3αl1	MASELEPEVQ	AI D- RSLLEC	SAEEI AGKWL	QATDLTREVY	OHL AHY VPKI	49
15	mouse_E3αl	MADEEMDGAE	RIMDVSPEPPL	AP OR PAS WMD	QQVDFYTAFL	HHL AQL VPEI	50
7	human_E3αl	MADEEAGGTE	RMEI SAEL PO	TPQRLAS WWD	QQV DF YT AF L	HHL AQL VPE	50
	Consensus	MA. E	MA. E D L	W.	Q D	. HLA VP. I	50
9	mouse_E3all	YCRGPNPFPQ	KEDT L'AQHI L	L GP ME WYI CA	E DP AL GF P K L	EQANKPSHLC	66
4	human_E3αl1	YCRGPNPFPQ	KEDML AQHVL	L GP ME WY L C'G	E DP AF GF P K L	EQANKPSHLC	66
15	mouse_E3 α l	Y F A E MDP DL E	K QE E S V QMS I	LTPLEWVLFG	EDPDI CLEKL	KHSG- AFOLC	66
7	human_E3αl	YF AE MDP DL E	KQEESVQMSI	FTPLEWYLFG	EDPDI CLEKL	KHSG- AFQLC	99
	Consensus	Y P	ж	L. P. EWYL. G		LC	100
				٠			
9	mouse_E3αII	GRVFKVGEPT	YSCRDCAVDP	TCVLCMECFL GSI HRDHRYR	GSI HRDHRYR	MITSGGGFC	149
4	human_E3αII	GRVFKVGEPT	YSCRDCAVDP	TCVLCMECFL	GSI HRDHRYR	MTTSGGGGFC	149
15	mouse_E3αl	GKVFKSGETT	YSCRDCAI DP	TCVL CMDCFQ	SSVHKNHRYK	MHTSTGGGFC	149
7	human_E3αl	GRVFKSGETT	YSCRDCAI DP	TCVLCMDCFQ	DSVHKNHRYK	MHT STGGGF C	149
	Cons ens us	GRVFK. GE. T	YSCRDCA. DP	TCVLCM CF.	. S. H HRY.	M TS. GGGFC	.150
						X.	
9	mouse_E3αII	DCGDTEAWKE	GPYCOKHKLS	GPYCQKHKLS SSEVVEEEDP	L VHL S E DVI A	RTYNI FAI MF	199
4	human_E3αII	DCGDTEAWKE	GPYCQKHELN	TSELEEEEDP	LVHLSEDVIA	RTYNI FAI TE	199
15	mouse_E3al	DCGDTEAWKT	GPFCVDHEPG	RAGTTKESLH	- CPLNEEVI A	QARRI FPSVI	198
7	human_E3αl	DCGDTEAWKT	GPFCVNHEPG	GPFCVNHEPG RAGTI KENSR	- CPLNEEVI V	QARKI FPSVI	198
	Cons ens us	DCGDT E AWK.	GP. C HE	GP. C HE L. E. VI A I F	L . E . VI A	-	200



Figure 1B

					•		
snau	mouse_E3aII	RYAVDI LTWE	KESELPEDLE	RYAVDILTWE KESELPEDLE VAEKSDTYYC MLFNDEVHTY	ML F NDE VHT Y	EQVI YTLQKA	249
mnu	human_E3αII	RYAVEI LTWE	KESELPADLE	KESELPADLE MVEKSDTYYC MLFNDEVHTY	ML F NDE VHT Y	EQVI YTLQKA	249
snou	mouse_E3αl	KYI VE MTI WE	EEKELPPELQ	KYI VEMTI WE EEKELPPELO I REKNERYYC VLFNDEHHSY	VL F NDE HHS Y	DHVI YSLQRA	248
שות	human_E3αl	KYVVEMTI WE	EEKELPPELQ	KYVVEMTI WE EEKELPPELQ I REKNERYYC VLFNDEHHSY	VL F NDE HHS Y	DHVI YSLQRA	248
Con	Cons ens us	. Y. VE WE	. E. ELP L.	. Y. VE WE . E. ELP L EK YYC . LFNDE. H. Y VI Y. LQ. A	. LFNDE. H. Y	VI Y. LQ. A	250
n ou	mouse_E3aII	VNCT QKE AI G	FATTVDRDGR	VNCTQKEAI G FATTVDRDGR RPVRYGDFQY CDQAKTVI VR NTSRQTK-PL	CDQAKTVI VR	NTSRQTK-PL	298
nnc	human_E3αII	VNCT QKE AIG	FATTVDRDGR	VNCT QKE A I G FATT V DR DGR RS V RYGDF QY		CEQAKSVI VR. NTSRQTK- PL	298
nαι	mouse_E3αl	LDCELAEAQL	HTTA! DKEGR	HTTAI DKEGR RAVKAGVYAT CQEAKEDI KS HSENVSQHPL	CQE AKE DI KS	HS E NV S QHP L	298
nuπ	human_E3αí	LDCELAEAQL	HTTA! DKEGR	LDCELAEAQL HTTAI DKEGR RAVKAGAYAA CQEAKEDI KS HSENVSQHPL	CQEAKEDI KS	HS E NV S QHP L	298
Con	Cons ens us	C EA	T D GR	T D GR R. V G C AK I PL	C AK I	PL	300
u au	mous e_E3aII	KVQVMHSSVA	KVQVMHSSVA AHQNFGLKAL	S W GS VI GY S	DGL RRI LCOV	SW.GSVI GYS DGLRRI LCQV GLQEGPDGEN	348
ηnι	huma n_ $E3\alpha II$	KVQVMHSSI V	KVQVMHSSI V AHQNFGLKLL	S W GS I I GYS	DGL RRI L C QV	SWLGSIIGYS DGLRRILCQV GLQEGPDGEN	348
nou	mous e_E3al	HVEVLHSVVM	AHQKF AL RL G	HVEVLHSVVM AHQKFALRLG SWMNKI MSYS SDFRQI FCQA CLVEEPGSEN	SDFRQI FCQA	CLVEEPGSEN	348
חשר	human_E3αl	HVEVLHSEI M	AHQKFALRLG	S WANK! MS Y S	S DF RQI F C QA	HVEVLHSEI M AHQKFALRLG SWMNKI MSYS SDFRQI FCQA CLREEPDSEN	348
Con	Cons ens us	. V. V. HS	AHQ. F. L. L.	SWIYS	R. I . CQ.	. V. V. HS AHQ. F. L. L. S.W I YS R. I . CQ L. E. P.D. E.N	350



Figure 1C

45(_ I/\>	Z	VO ET PTIA	T INS N I I A B I I DI I DI I DI I DI I DI I DI		
448	SVI TETLLEV	RHLI EEONVI	VQMFTVPTLA	LOKEYI SDDH DRSI SI TALS VOMFTVPTLA RHLI EEQNVI SVI TETLLEV	human_E3αl	7
448	SVITETLLEV	RHLI EEQNVI	VOMLTVPTLA	LOKEYISDDH ERSISITALS VOMLTVPTLA RHLIEEQNVI SVITETLLEV	mouse_E3 α l	15
448		RMLITEENLM	VOFFTAPTLA	LORDFMEDDH ERAVSVTALS VOFFTAPTLA RMLITEENLM SIIIKTFMDH	human_E3αII	4
448	TVI I KAF MDH	RMLLTEENLM	VQFFTAPTLA	LORDFMEDDH ERAVSVTALS VOFFTAPTLA RMLLTEENLM TVIIKAFMDH	mo us e_E3αII	9
40(A. F. K. Y. Q	. M YKKLF	H. L SS.	L RLML. D. KL. KGAR H. L SS M YKKLF A F. K. Y. Q	Consensus	
398	AMEFVKYYKQ	FWEMEYKKLF	I LHELI FSSF	PCLISRLMLW DAKLYKGARK ILHELIFSSF FMEMEYKKLF AMEFVKYYKQ	human_E3αl	7
398	AMEFVKYYKQ	FMEMEYKKLF	I L HELI FSSF	PCLISRLMLW DAKLYKGARK ILHELIFSSF FMEMEYKKLF AMEFVKYYKO	mous e_E3αl	15
368	AVRFAKNYQQ	LMDLKYKKLF	VY HQL F MS S L	SSLVDRLMLS DSKLWKGARS VYHQLFMSSL LMDLKYKKLF AVRFAKNYQQ	human_E3αII	4
398	ALRFAKNYRQ	LMDLKYKKLF	VYHQLFMSSL	SSLVDRLMLN DSKLWKGARS VYHQLFMSSL LMDLKYKKLF ALRFAKNYRQ	mouse_E3aII	9



Figure 1D

SEO	SEQ I D NO:						
9	mouse_E3 α II	LKHRDAQGRF	QFERYTAL QA FKFRRVQSLI	FKFRRVQSLI	L DL KYVLI SK	LDLKYVLI SK PTEWSDELRO	498
4	human_ $E3\alpha II$	LRHRDAQGRF	OFERYTAL QA FKFRRVQSLI	FKFRRVQSLI	L DL KYVLI SK	LDLKYVLI SK PTEWSDELRO	498
15	mouse_E3∝l	LPEYLDRNN-	KEN-FOGYSQ DKLGRVYAVI	DKL GRVY AV I	CDLKYI LI SK	CDLKYILISK PVI WTERLRA	496
7	human_E3∝l	LPEYLDRNN-	KFN- FQGYSQ DKLGRVYAVI	DKL GRVY AVI	CDLKYI LI SK	CDLKYI LI SK PTI WTERLRM	496
	Consensus	L	. F	. K RV I	. DLKY. LI SK PT. W LR.	PT. W LR.	500
9	mouse_ $E3\alpha II$	KFL QGF DAFL	ELLKCMQGMD PI TRQVGQHI	PI TROVGOHI	EMEPEWEAAF	TLOMKLTHVI	548
4	human_E3αII	KFLEGFDAFL	ELLKCMOGMD PI TROVGOHI	PI TROVGOHI	E ME P E WE AA F	TLOMKLTHVI	548
15	mouse_E3αl	QFLEGFRSFL	KI LTCMQGME	EI RRQVGQHI	EVDPDWEAAI	AI QMOLKNI L	546
2	human_E3αl	OFLEGFRSFL	KILTCMQGME	EI RRQVGQHI	EVDPDWEAAI	AI QMQLKNI L	546
	Cons ens us	. FLEGF FL	. FLEGF FL L. CMQGM	. I . RQVGQHI	E P. WE AA.	OM L	550
9	mous e_E3 α II	S MV QDWCALD	S MY ODWCALD EKYLIEAYKK CLAVLTQCHG GFTDGEQPIT LSICGHS VET	CLAVLTQCHG	GFT DGE QPI T	LSI CGHS VET	598
4	human_ $E3\alpha II$	S MWQDWC A:S D	SMMODWCASD EKVLIEAYKK CLAVLMQCHG GYTDGEQPIT LSICGHSVET	CL AVL MQCHG	GYT DGE OPI T	LSI CGHSVET	598
15	mouse_E3al	L MF QE WCACD	LMF QEWCACD EDLLLVAYKE	CHKAVMRCST NFMSSTKTV-	NF MS S T K T V-	VQL CGHS L ET	595
7	human_E3αl	L MF QE WCACD	L MF QE WCACD EELLLVAYKE	CHKAVMRCST SFISSSKTV-	SFI SSSKTV-	VQS CGHS L ET	. 595
	Consensus	. M Q. WCA. D	. M Q. WCA. D E L AYK. C M C F	C M. C		CGHS. ET	9



Figure 1E

648 648 645 645	698 698 695 700	748 748 738 738
PLSELSPPML PLSELSPPML PFDSFQVEVL SFEDFQVEVL PL	YYYHNVKCRR EMFDKDI VML YYYHNVKCRR EMFDKDVVML FYYQDVKCRE EMYDKDI I ML FYYQDVKCRE EMYDKDI I ML YYVKCR. EM.DKDI I ML	HKDVVQQNNT HKDVVQQNNT DQDL I KQYNT DQDL I KQYNT
EVAYKFPELL EVAYKFPELL GAI SRLHEFV GAVSRLHEFVE	YYYHNVKCRR EMFDKDI VML YYYHNVKCRR EMFDKDVVML FYYQDVKCRE EMYDKDI I ML FYYQDVKCRE EMYDKDI I ML YYVKCR. EM. DKDI I ML	QTGVSMMDPN HFLM MLSRF ELYQLFSTPD YGKRFSSEVT HKDVVQQNNT OTGVSMMDPN HFLM MLSRF ELYQIFSTPD YGKRFSSEIT HKDVVQQNNT QIGASI MDPN KFLLLVLQRY ELTDA FNKTISTK DQDLIKQYNT QIGASLMDPN KFLLLVLQRY ELAEA FNKTISTK DQDLIKQYNT
IRYCVSQEKV SIHLPISRLL AGLHVLLSKS EVAYKFPELL PLSELSPPMLIYCVSQEKV SIHLPVSRLL AGLHVLLSKS EVAYKFPELL PLSELSPPMLKSYKVSEDLV SIHLPLSRTL AGLHVRLSRL GAISRLHEFV PFDSFQVEVLKSYRVSEDLV SIHLPLSRTL AGLHVRLSRL GAVSRLHEFV SFEDFQVEVLXSYRVSEDLV SIHLP.SR.L AGLHV.LS	I EHPLRCLVL CAQVHAGMMR RNGFSLVNQI YYYHNVKCRR EMFDKDI VMLIEHPLRCLVL CAQVHAGMMR RNGFSLVNQI YYYHNVKCRR EMFDKDVVMLVEYPLRCLVL VAQVVAEMMR RNGLSLI SQV FYYQDVKCRE EMYDKDI I MLVEYPLRCLVL VAQVVAEMMR RNGLSLI SQV FYYQDVKCRE EMYDKDI I MLVEYPLRCLVL VAQVVAEMMR RNGLSLI SQV FYYQDVKCRE EMYDKDI I MLVEYPLRCLVL AQV. A. MMR RNG. SL Q YY VKCR. EM DKDI . ML	QTGVSMMDPN HFLM MLSRF ELYQLFSTPD YGKRFSSEVT HKDVVQQNNT QTGVSMMDPN HFLM MLSRF ELYQI FSTPD YGKRFSSEIT HKDVVQQNNT QI GASI MDPN KFLLLVLQRY ELTDA FNKTI STK DQDLI KQYNT QI GASLMDPN KFLLLVLQRY ELAEA FNKTI STK DQDLI KQYNT
SI HLPI SRLL I HLPVSRLL A SI HLPLSRTL SI HLPLSRTL SI HLPLSRTL	I EHPLRCLVL CAQVHAGMMR RNGFSLVNQI I EHPLRCLVL CAQVHAGMMR RNGFSLVNQI VEYPLRCLVL VAQVVAEMMR RNGLSLI SQV VEYPLRCLVL VAQVVAEMMR RNGLSLI SQV . E. PLRCLVL . AQV. A. MMR RNG. SL Q.	HFLMI MLSRF HFLMI MLSRF KFLLLVLQRY KFLLLVLQRY
I RYCVSQEKV I YCVSQEKV S KSYKVSEDLV KSYRVSEDLV Y.VSV	I EHPLRCLVL I EHPLRCLVL VEYPLRCLVL VEYPLRCLVL . E. PLRCLVL	QT GVS MWDP N QT GVS MWDP N QI GAS I MDP N QI GAS L MDP N
mous e_E3αII human_E3αII mous e_E3αI human_E3αI Cons ens us	mo us e_E3αII h u ma n_E3αII mo us e_E3αI h u ma n_E3αI Co ns e ns us	$muse_E3\alpha II$ $human_E3\alpha II$ $muse_E3\alpha I$ $human_E3\alpha I$
6 4 2 2	6 4 1 2	6 4 1 2



Figure 1F

006	VN. L. CDVM	SSKAE Q. KQ D. ALPPP P. FCP. F VN. L. CDVM	. D. ALPPP	. O. K O.	SSKAE.	Consensus	
888	I NL L NCDI MM	SKTOHSKAEH MOKKRRKOEN KDEALPPPPP PEFCPAFSKV I NLLNCDI MM	KDEALPPPPP	MOKKRRKQEN	S K T QHS K A E H	human_E3αl	7
888	VNL LSCDVM		MQKKRRKQEN KDEALPPPPP PEFCPAFSKV		SKTQHSKAEH	mouse_E3αl	15
868	VNI LQS DVML	PPFCPLFASL	EDTALPPPVL	AQRKL KRQNR	SRAEOSKAEE	human_E3αII	4
868	VNI LOCDVML	PPFCPLFASL	EDTALPPPAL	AQRKLKRENK	SRAEQSKAEE	mous e_E3aII	9
850	K. FN. YFYH.	. LPE. EN. ET G. E. VI VA. FKKPGG. G. YELK. E	A . FKKPG G.	G. E. VI V	. LPE. EN. E ⁻	Cons ens us	
838		NLPENENNET GLENVINKVA TFKKPGVSGH GVYELKDESL	A TFKKPGVSG	GLENVI NKV	NL PENENNE ⁻	human_E3αl	7
838	K D F N M Y F Y H Y	NLPENENNET GLENVINKVA TFKKPGVSGH GVYELKDESL KDFNMYFYHY	. TFKKPGVSGH	GLENVI NKVA	NL PENENNET	mouse_E3αl	15
848	KEFNLYFYHF	SLPEDENKET GMESVIEAVA HFKKPGLTGR GMYELKPECA KEFNLYFYHF	A HFKKPGLTGR	GMESVIEAVA	S L P É DE NKE 1	human_ $E3\alpha II$	4
848	KEFNLYFYHF	SLPEDENKET GMESVIESVA HFKKPGLTGR GMYELKPECA KEFNLYFYHF	A HFKKPGLTGR	GMESVIESVA	SLPEDENKET	mous e_E3αII	9
800	. PM HS K	. REI I H. L. I	VGER PG VG. V		LI EEMLI	Consensus	
788	EP MP HS AI AK	. MREIIHLLCI	YI VGERYVPG VGNVTKEEVT		LI EEMLOVLI	human_E3αl	2
788	EPMPHSAI AR	MREI THLLCI	YI VGERYVPG VGNVTREEVI	YI VGERYVPG	LI EEMLQVLI	muse_E3αl	15
798	KP MAHS EL VK	KREI I HOLSI	MLVGERFSPG VGQVNATDEI		LIEEMLYLII	human_E3αII	4
798	KP MAHS EL V K	KREI I HQL SI	3 VGQVAAT DEI	LIEEMLYLII MLVGERFNPG VGQVAATDEI	LIEEMLYLII	mouse_E3aII	9



Figure 1G

SEQ	SEQ I D NO:						
9	mouse_E3aII	YI MGTI LQWA	VEHHGSAWSE SMLQRVLHLI	S ML QRVL HLI	GMAL QE E K HH	GMALQEEKHH LENAVEGHVQ	948
4	human_E3αII	CI MGTI LQWA	V E HNGY AWS E	S ML QR VL HL I	GMALQEEKQH LENVTEEHVV	LENVTEEHVV	948
15	mouse_ $E3\alpha$ l	YI LRTI FERA	YILRTI FERA VDTESNLWTE GMLQMAFHIL	GML QMAFHI L	ALGLLEEKQQ LQKAPEEEV-	L QKAPEEEV-	937
7	human_E3 α l	YILRTVFERA	YILRTVFERA IDTDSNLWTE GMLQMAFHIL	GML QMAF HILL	ALGLLEEKQQ LQKAPEEEV-	L QK APEEEV-	937
	Consensus	YI TI A	YI TI A V W. E . MLQ H L. EEKQ. L. A. EE. V.	. ML Q H	L. EEKQ.	L A. EE. V.	950
			7				
9	mouse_ $E3\alpha II$	TFTFTQKI SK	TFTFTQKISK PGDAPHNSPS ILAMLETLQN APSLEAHKDM IRWLLKMFNA	ILAMLETLON	APSLEAHKDM	I RWLLKMFNA	866
4	human_E3αII	TFTFTQKI SK	TFTFTQKISK PGEAPKNSPS ILAMLETLQN APYLEVHKDM IRW LKTFNA	ILAMLETLON	APYLEVHKDM	I RW LKTFNA	866
15	mo us e_ $E3\alpha$ l	AF DF Y HKAS R	AFDFYHKASR LGSSAMNAQN I QMLLERLKG I PQLEGQKDM I TWILQMFDT	IOMLLERLKG	I PQL EGQKDM	I TW LOMFDT	987
7	huma n_E3 α l	TFDFYHKASR	TFDFYHKASR LGSSAMNIOM LLEKLKG IPQLEGOKDM ITW LOMFDT	LLEKLKG	I PQLEGQKDM	I TW LOWF DT	984
	Cons ens us	TF. F K. S.	TF. F K. S G N I LE. L P. LE KDM I . M L. MF	ILE.L	. P. LE KDM		1000
9	mous e_ $E3\alpha II$	I KKI RECS	IKKIRE CS SSSPVAEAEG TIMEESSRDK DKAERKRKAE IARLRREKIM 1046	TI MEESSRDK	DKAERKRKAE	I ARL RREKI M	1046
4	human_E3al	VKKMRE SS	PTSPVAETEG	PTSPVAETEG TIMEESSRDK	DKAERKRKAE	I ARLRREKI M 1046	1046
15	mouse_E3αl	VKRLREKSCL	VVATTSGLEC	VVATTSGLEC I KSEEI THDK	EKAERKRKAE	AARLHRQKI M 1037	1037
7	huma′n_E3αl	VKRLREKSCL		I KNDEI THDK	EKAERKRKAE	I VATTSGSES I KNDEI THDK EKAERKRKAE AARLHRQKI M 1034	1034
	Consens us	VK RE C.	VK RE C E EE DK . KAERKRKAE . ARL. R. KI M 1050	EE DK	. KAERKRKAE	. ARL. R. KI M	1050



Figure 1H

6 1 2 2	mouse_E3αII human_E3αII mouse_E3αI human_E3αI Consensus	AQMS E MQR HF I DE NKEL F QQ AQMS E MQR HF I DE NKEL F QQ AQMS AL QK NF I ET HKL MY DN AQMS AL QK NF I ET HKL MY DN AQMS . Q. F I K	AQMSEMQRHF I DENKELFQQ TLELDTSASA TLDSSPPV SDAALTALGP 1094 AQMSEMQRHF I DENKELFQQ TLELDASTSA VLDHSPVA SDMTLTALGP 1094 AQMSALQKNF I ETHKLMYDN TSEVTGKEDS I MEEESTSAV SEASRI ALGP 1087 AQMSALQKNF I ETHKLMYDN TSEMPGKEDS I MEEESTPAV SDYSRI ALGP 1084 AQMS. Q. F I K T.E	SDAALTALGP SDMTLTALGP SEASRIALGP SDYSRIALGP SDALGP	1094 1094 1087 1084 1100
6 4 7 2 2 2 2	mouse_E3αII human_E3αII mouse_E3αI human_E3α Consensus	AQTQVPEPRQ FVTCI LCQEE TQTQVPEQRQ FVTCI LCQEE KRGPAVTEKE VLTCI LCQEE KRGPSVTEKE VLTCI LCQEE	AQTQVPEPRQ FVTCI LCQEE QEVTVGSRAM VLAAFVQRST VLSKDRTKTI TQTQVPEQRQ FVTCI LCQEE QEVKVESRAM VLAAFVQRST VLSKNRSKFI KRGPAVTEKE VLTCI LCQEE QEVKLENNAM VLSACVQKST ALTQHRGKPV KRGPSVTEKE VLTCI LCQEE QEVKI ENNAM VLSACVQKST ALTQHRGKPI		1144 11144 11137 1134
6 1 5 2	mous e_E3aII huma n_E3aII mous e_E3aI huma n_E3aI Cons ens us	AD- PEKYDPL FWHPDLSCGT QD- PEKYDPL FWHPDLSCGT DHLGETLDPL FWDPDLAHGT ELSGEALDPL FWDPDLAYGT	HTGSCGHVMH AHCWQRYFDS HTSSCGHI MH AHCWQRYFDS YTGSCGHVMH AVCWQKYFEA YTGSCGHVMH AVCWQKYFEA TGSCGHVMH A.CWQ.YF	VQAKEQRRQQ 1193 VQAKEQRRQQ 1193 VQLSSQQ 1184 VQLSSQQ 1181 VQQQ 1200	1193 1193 1184 1181



Figure 11

1241 1241 1233 1230 1250	1290 1290 1283 1279 1300	1340 1340 1333 1329
RLRLHTSYDV ENGEFLCPLC ECLSNTVIPL L-LPPRSILS RRLN-FSDQP 1241 RLRLHTSYDV ENGEFLCPLC ECLSNTVIPL L-LPPRNIFN NRLN-FSDQP 1241 RIHVDL-FDL ESGEYLCPLC KSLCNTVIPI IPLQPQKINS ENAEALAQLL 1233 RIHVDL-FDL ESGEYLCPLC KSLCNTVIPI IPLQPQKINS ENADALAQLL 1230 RD. E. GE. LCPLCL. NTVIPL. PI. S	DLAQWTRAVT QQI KVVQMLR RKHNAA-DTS SSEDTEAMNI IPI PEGFRPD 1290 NLTQWIRTIS QQI KALQFLR KEESTP-NNA STKNSENVDE LQLPEGFRPD 1290 TLARW QTVL ARI SGYNI KH AKGEAPAVPV LFNQGMGDST FEFHSI LSFG 1283 TLARW QTVL ARI SGYNI RH AKGENP-I PI FFNQGMGDST LEFHSI LSFG 1279 LA.WI.TV	
L - L PPRSI LS L - L PPRNI FN I PL QPQKI NS I PLQPQKI NS I PLQPQKI NS	S S E DT E AMNI S T K NS E NV DE L F NQGMGDS T F F NQGMGDS T	AAYKVGLKVH PNEGDPRVPI LCWGTCAYTI ATYKVGLKVH PNEEDPRVPI MCWGSCAYTI TIYRIGLKVP PDELDPRVPM MTWSTCAFTI TIYRIGLKVP PDERDPRVPM LTWSTCAFTI YGLKV. P.E.DPRVPW.TCA.TI
ECLSNTVI PL ECLSNTVI PL KSLCNTVI PI KŠLCNTVI PI L. NTVI P.	QQI KVVQMLR RKHNAA-DTS SSEDTEAMNI QQI KALQFLR KEESTP-NNA STKNSENVDE ARI SGYNI KH AKGEAPAVPV LFNQGMGDST ARI SGYNI RH AKGENP-1 PI FFNQGMGDST	FYPRNPYSDS I KEMLTTFGT AAYKVGLKVH PNEGDPRVPI FRPKI PYSES I KEMLTTFGT ATYKVGLKVH PNEEDPRVPI VQSSVKYSNS I KEMVI LFAT TI YRI GLKVP PDELDPRVPM VESSI KYSNS I KEMVI LFAT TI YRI GLKVP PDERDPRVPM
ENGEFLCPLC ECLSNTVIPL ENGEFLCPLC ECLSNTVIPL ESGEYLCPLC KSLCNTVIPI ESGEYLCPLC KSLCNTVIPI E. GE. LCPLC L. NTVIP.	QQI KVVQMLR QQI KALQFLR ARI SGYNI KH ARI SGYNI RH	FYPRNPYSDS I KEMLTTFGT FRPKI PYSES I KEMLTTFGT VQSSVKYSNS I KEMVI LFAT VESSI KYSNS I KEMVI LFAT VESSI KYSNS I KEMVI LFAT THE STAN STAN STAN STAN STAN STAN STAN STAN
RLRLHTSYDV RLRLHTSYDV RI HVDL - FDL RI HVDL - FDL R D.	DLAQWTRAVT NLTQW RT! S TLARW QTVL TLARW QTVL . LA. W. TV.	FYPRNPYSDS I KEMLTTFGT FRPKI PYSES I KEMLTTFGT VQSSVKYSNS I KEMVI LFAT VESSI KYSNS I KEMVI LFAT
mous e_E3αII huma n_E3αII mous e_E3αI huma n_E3αI Cons ens us	mous e_E3αII huma n_E3αII mous e_E3αI huma n_E3αI Cons ens us	mous e_E3αII huma n_E3αII mous e_E3αI huma n_E3αI Cons ens us
2 1 5	6 4 4 7 2 2 2 2 3 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	6 4 15 2



Figure 1J

SEQ ID NO:						
6 mouse_E3aII	QSIERILSDE	EKPVFGPLPC	RLDDCLRSLT	RFAAAHWTVA	LLPVVQGHFC	1390
4 human_E3αII	QSIERILSDE	DKPLFGPLPC	RLDDCLRSLT	RFAAAHWTVA	SVSVVQGHFC	1390
15 mouse_ $E3\alpha I$	QAIENLLGDE	GKPLFGALQN	RQHSGLKALM	QFAVAQRATC	PQVLIHKHLA	1383
2 human_E3αI	QAIENLLGDE	GKPLFGALQN	RQHNGLKALM	QFAVAQRITC	PQVLIQKHLV	1379
Consensus	Q . IE L .DE	Q.IEL.DE .KPLFG.L	$R \ldots L \ldots L .$. FA . A	Q . H	1400
6 mouse_E3aII	KLFASLVPSD	SYEDLPCILD	IDMFHLLVGL	VLAFPALQCQ	DFSGSSL	1437
4 human_E3αII	KLFASLVPND	· SHEELPCILD	IDMFHLLVGL	VLAFPALQCQ	DFSGISL	1437
15 mouse_ $E3\alpha I$	RLLSVILPNL	QSENTPGLLS	VDLFHVLVGA	VLAFPSLYWD	DTVDLQPSPL	1433
2 human_E3αI	RLLSVVLPNI	KSEDTPCLLS	IDLFHVLVGA	VLAFPSLYWD	DPVDLQPSSV 1429	1429
Consensus	. L PN.	E PC .L .	ID. FH. LVG .	VLAFP. L	D SSL	1450
6 mouse_E3αII	ATGDLHIF	HLVTMAHIVQ	ILLTSCTEEN	GMDQENP	TGEEELAILS	1482
4 human_E3αII	GTGDLHIF	HLVTMAHIIQ	ILLTSCTEEN	GMDQENP	PCEEESAVLA	1482
15 mouse_E3aI	SSSYNHLYLF	HLITMAHMLQ	ILLTTDTDLS	PGPPLAEGEE	DSEEARCASA	1483
2 human_E3αI	SSSYNHLYLF	HLITMAHMLQ	ILLTVDTGL-	PLAQVQE	DSEEAHSASS 1475	1475
Consensus	F	HL. TMAH Q ILLTT	ILLTT	0	EE	1500



Figure 1K

6 mouse_E3αII 4 human_E3αII 15 mouse_E3αI 2 human_E3αI Consensus	LHKTLHQYTG LYKTLHQYTG FFVEVSQHTD FFAEISQYTS	SALKEAPSGW SALKEIPSGW GLTGCGAPGW GSIGCDIPGW	SALKEAPSGW HLWRSVRAAI MPFLKCSAL SALKEIPSGW HLWRSVRAGI MPFLKCSALF GLTGCGAPGW YLWLSLRNGI TPYLRCAALL GSIGCDIPGW YLWVSLKNGI TPYLRCAALFGW . LW. S. R. GI . P. L. C. ALF	MPFLKCSAL MPFLKCSALF TPYLRCAALL TPYLRCAALF . P. L. C. ALF	FHYLNGVPAP 1532 FHYLLGVAPP 1532 FHYLLGVAPP 1533 FHYLLGVTPP 1525 FHYL.GVP 1550
6 mouse_E3αII 4 human_E3αII 15 mouse_E3αI 2 human_E3αI Consensus	PDLQV-SGTS PDIQV-PGTS EELFANSAEG EELHTNSAEG	HFEHLCNYLS HFEHLCSYLS EFSALCSYLS EYSALCSYLS . F LCSYLS	LPTNLIHLFQ ENSDIMNSLI LPNNLICLFQ ENSEIMNSLI LPTNLFLLFQ EYWDTIRPLL LPTNLFLLFQ EYWDTVRPL	LPTNĻIHLFQ ENSDIMNSLI LPNNLICLFQ ENSEIMNSLI LPTNLFLLFQ EYWDTIRPLL LPTNLFLLFQ EYWDTVRPLL LPTNLLFQ EDL.	LPTNLIHLFQ ENSDIMNSLI ESWCQNSEVK 1581 LPNNLICLFQ ENSEIMNSLI ESWCRNSEVK 1581 LPTNLFLLFQ EYWDTIRPLL QRWCGDPALL 1583 LPTNLFLLFQ EYWDTVRPLL QRWCADPALL 1575 LPTNLLFQ EDLWC 1600
6 mouse_E3all 4 human_E3all 15 mouse_E3al 2 human_E3al Consensus	RYLNGERGAI RYLEGERDAI KSLKQKSAVV NCLKQKNTVV	SYPRGANKLI RYPRESNKLI RYPRKRNSLI RYPRKRNSLI RYPRN.LI	DLPEDYSSLI NLPEDYSSLI ELPEDYSCLL ELPDDYSCLL	NQASNFSCPK NQASNFSCPK NQASHFRCPR NQASHFRCPR NQAS.F.CP.	NQASNFSCPK SGGDKSRAPT 1631 NQASNFSCPK SGGDKSRAPT 1631 NQASHFRCPR SADDERKHPV 1633 NQASHFRCPR SADDERKHPV 1625 NQAS.F.CP. SDP. 1650



Figure 1L

x 4	n 0 -		m	2	0					
168 168 168	170	173	173	1725	1750					
CLVCGSLLC SQSYCCQAEL EGEDVGACTA HTYSCGSGAG IFLRVRECQV 1681 CLVCGSLLC SQSYCCQTEL EGEDVGACTA HTYSCGSGVG IFLRVRECQV 1681 CLFCGAILC SQNI CCQEIV NGEEVGACVF HALHCGAGVC IFLKI RECRV 1683	CL.CGLC SQCCQ GE.VGAC. HCG.GV. IFL. RECKV 1073 CL.CGLC SQCCQ GE.VGAC. HCG.GV. IFL. REC.V 1700	FLAGKTKGC FYSPPYLDDY GETDQGLRRG NPLHLCKERF KKI QKLWHQH 1731	LVEGKARGC AYPAPYLDEY GETDPGLKRG NPLHLSRERY RKLHLVWQQH 1733	VLVEGKARGC AYPAPYLDEY GETDPGLKRG NPLHLSRERY RKLHLVWQQH	GK GC . Y PYLD. Y GETD. GL. RG NPLHL ER. RK WQQH					
HTYSCGSGAG HTYSCGSGVG HALHCGAGVC	HAL H. GG. GV.	NPL HL CKERF	NPLHLSRERY	NPLHLSRERY	. NPLHLER.	1755	1755	1757	1749	1774
EGEDVGACTA EGEDVGACTA NGEEVGACVE	GE. VGAC	GET DQGL RRG	GETDPGLKRG	GETDPGLKRG	GETD. GL. RG		WQHL	WQL L	WQLL	MQ. L
S QS Y C C QAEL S QS Y C C QT E L S QNI C C QE I V	SQ. CCQ EVSPPVIDDY	FYSPPYLDDY	AYPAPYL DEY	AYPAPYLDEY	. Y PYLD. Y	I TEEI GHAQ EANQTLVGI D WQHL	VTEEI GHAQ EANQTLVGI D WQHL	CIIEEI ARSQ ETNOMLFGFN WOLL	CIIEEI ARSQ ETNOMLFGFN WOLL	. I . E E I Q E . NQ. L . G WQ. L
LCLVCGSLLC LCLVCGSLLC LCLFCGAI LC	LCL. CG LC	LFLAGKTKGC	VLVEGKARGC	VL VEGKARĞ C	GK GC	SI TEEI GHAQ	SVTEEI GHAQ	CI I EEI ARSQ	CI I EEI ARSQ	. I. EEI 0
mous e_E3aII huma n_E3aII mous e_E3aI	Consensus E321	human_E3αII	mous e_E3al	human_E3αl	Cons ens us	mouse_E3 αII	$human_E3\alpha II$	mouse_E3αl	human_E3αl	Consensus
6 1 5	7 4	> 4	15	2		9	4	15	7	



FIG. 2

Tth Expression Profile of huE3α–II

in Human Tissues

Brain
Heart
Skeletal muscle
Colon
Thymus
Spleen
Kidney
Liver
Small intestine
Placenta
Lung
Leukocyte

9.5kb —

7.5kb —

4.4kb —

2.4kb —



FIG. 3

Tth Expression Profile of huE3 α -l in Human Tissues

Heart Brain Placenta Lung Liver Skeletal Muscli Kidney

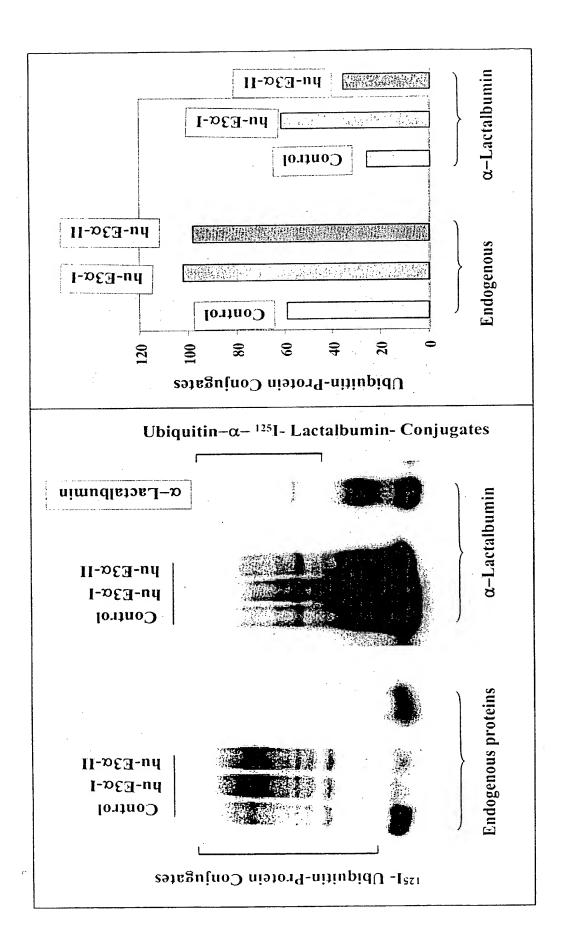


4.4kb -

2.4kb —



Figure 4
Ubiquitination of Endogenous Proteins

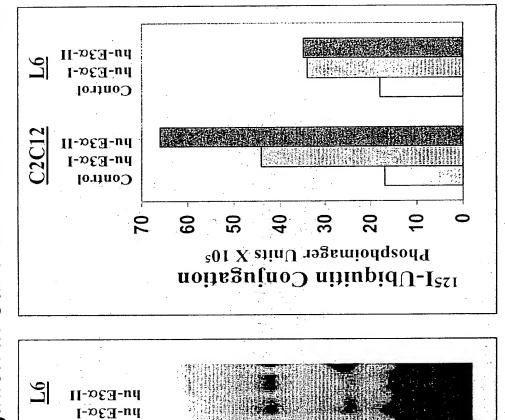


Fransfection of Human E3a-I or E3a-II cDNA Stimulates Ubiquitin Conjugation in Cultured Muscle Cell Lines Figure 5

Control

Control hu-E3a-I hu-E3a-II

C2C12



Conjugates

1251-Ubiquitin-Protein





Figure 6

 $^{125}\mbox{l-Ubiquitin Conjugation to Muscle Proteins and Its Sensitivity to E3}_{\alpha}$ Inhibitor in Skeletal Muscle Extracts

Free Ub	
1251-Uh-Protein Conjugates	
	+ Arg ME Tumor-5D
Contro v.s. 5-day tumor-bearing	(min) Tumor-5D Tumor-5D
t mmo	Tumor-5D Tumor-5D
S. S-day	Control Tumor-5D Control Tumor-5D Tumor-5D AAL AAL AAL AAL AAL AAL AAL A
ontro v.	Control Tumor-5D
الم الم	
	a
-day tumor-bearing	Control Tumor-3D Ario A.
umor-t	Tumor-3D Tumor-3D (Control Tumor-3D)
3-day t	ion Time (min)
	React Control Tumor-3D
Contro v.s	Control 7 Tumor-3D
səlegulno Conjugates	
Free Up	



Figure 7

Rates of Ubiquitination of N-end Rule Substrate α-Lactalbumin in Skeletal Muscle Extracts

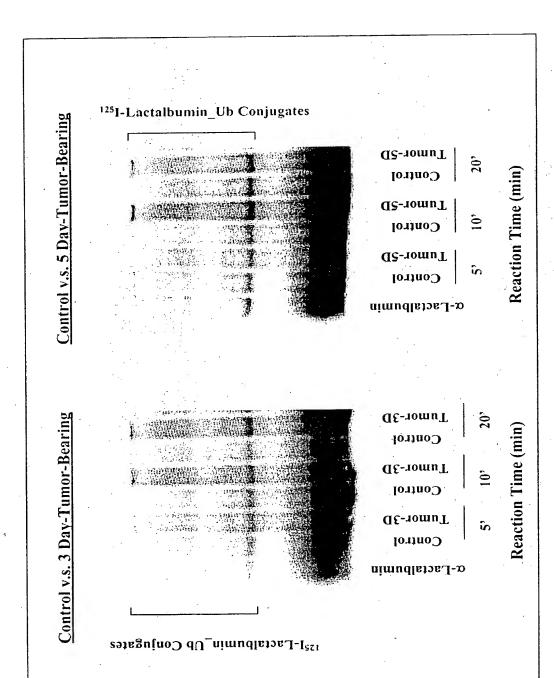
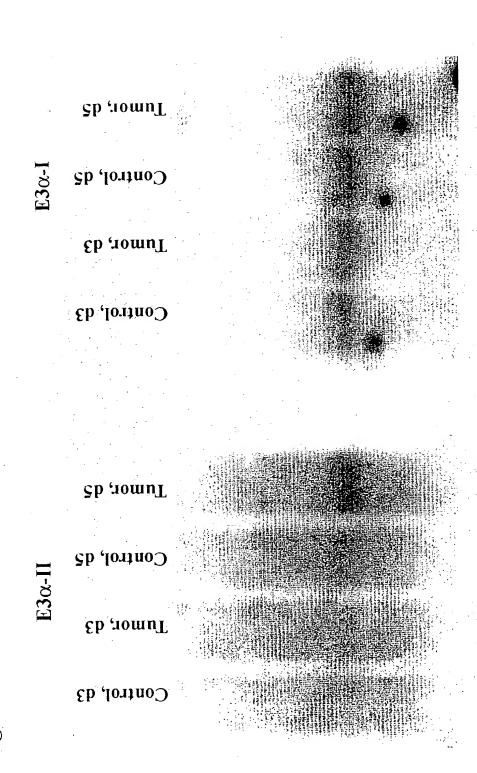


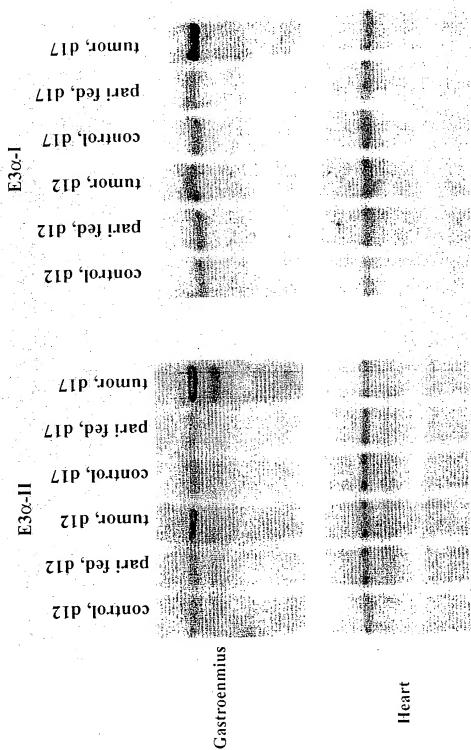


Figure 8

in gastrocnemius muscles in YAH-130 exprimental cachexia model Northern blot analysis of E3 α -I & E3 α -II expression



Northern blot analysis of E3 α -I and E3 α -II expression in gastrocnemius muscle and cardiac muscle in C26 experimental cachexia model



Heart



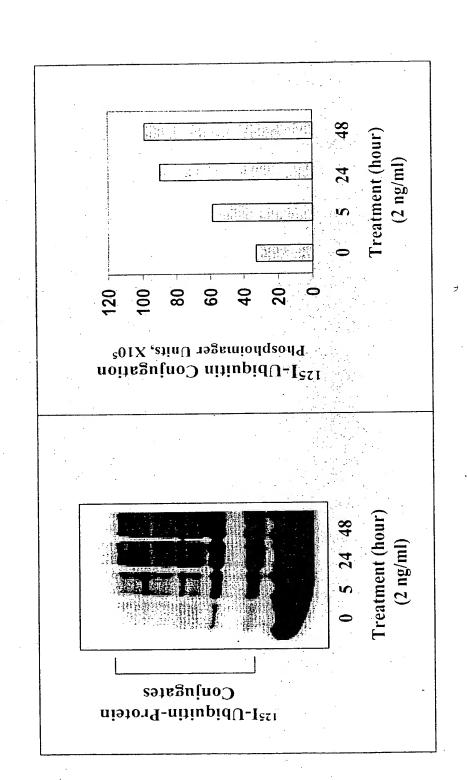
Figure 10

induce E3α-II Expression in C2C12 myostube culture Proinflammatory cytokines TNF- α and IL-6

5 days		
3 days		
1L-6 10 ng/m1		
5 days		
3 days		
TNF-a 10 ng/ml	Ε3α-Π	E3α-I

OIPE CZES

IL-6 Elicits Accelerated Ubiquitination in C2C12 Myotube Cultures Figure 11





 ${\sf TNF}_{\alpha}$ Elicits Accelerated Ubiquitination in C2C12 Myotube Cultures Figure 12

